PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
4239-68226-01		
International application No. PCT/US2004/017736	International filing date *(day/month/year)* 04.06.2004	Priority date *(day/month/year)* 05.06.2003
International Patent Classification (IPC) or national classification and IPC C07K14/32, A61K39/07, A61P31/04, C07K16/12		
Applicant THE GOVERNMENT OF THE UNITED STATES OF ... et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.03.2005	17.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Fausti, S Telephone No. +49 89 2399-7389

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/017736

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-47 as originally filed

Sequence listings part of the description, Pages

1-8 as originally filed

Claims, Numbers

1-33 received on 07.03.2005 with letter of 03.03.2005

Drawings, Sheets

1, 2 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/017736

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 17-20,31-33 (with respect to Industrial Applicability)

   because:

   ☒ the said international application, or the said claims Nos. 17-20,31-33 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6,9,21-33
	No:	Claims	1-5,7,8,10-20
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-33
Industrial applicability (IA)	Yes:	Claims	1-16,21-30
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item II
Priority

P.1 In view of the content of the earlier US application, the priority date of 05.06.2003 appears to be validly claimed. Accordingly, D6 is not considered as comprised in the state of the art (see below).

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

N.1 Claims 17-20 and 31-33 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT, namely methods of treatment of the human/animal body. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. DOCUMENTS and ABBREVIATIONS.

γ-PGA: poly-γ-glutamic acid;
γ-D-PGA: poly-γ-(D)-glutamic acid;
PA: Protective Antigen;
BSA: Bovine Serum Albumin;
KLH: Keyhole Limpet Hemocyanin.

Reference is made to the following documents:

D1: Alkan S. S. et Al., *Journal of Immunology* (1971) Vol. 107, No. 2, Pages 353-

358;

D2: Goodman J. W. et Al., *Biochemistry* (1968) Vol. 7, No. 2, Pages 706-710;

D3: Klaus G. G. et Al., *European Journal of Immunology* (1975) Vol. 5, No. 2, Pages 105-111;

D4: Senyk G. et Al., *Immunochemistry* (1972) Vol. 9, No. 2, Pages 97-110;

D5: Emmanuel J.-p. & Prodhomme F., *Abstracts of Papers American Chemical Society* (2000) Vol. 219, No. 1-2, Page BIOL133;

D6: Schneerson R. et Al., *PNAS* (2003) Vol. 100, No. 15, Pages 8945-8950;

D7: Leppla S. H. et Al., *Journal of Clinical Investigation* (2002) Vol. 110, No. 2, Pages 141-144;

D8: Welkos S. et Al., *Microbiology* (2001) Vol. 147, No. 6, Pages 1677-1685.

D9: WO 01/60412 A.

1.1 D1 discloses conjugates of γ-D-PGA from B. anthracis and (L)-Tyrosine-azobenzenearsonate eliciting anti-PGA antibodies in guinea pigs challenged with the conjugates (see: abstract; page 354, right-hand column, second paragraph; paragraph joining pages 355 and 356; page 356, paragraph joining left- and right-hand column; page 357, left-hand column, first paragraph).

1.2 D2 discloses rabbit antisera against the γ-PGA capsular polypeptide of B. anthracis prepared by immunization with intact bacilli or the capsular polypeptide complexed with methylated BSA (see: abstract; page 706; right-hand column, lines 10-13).

1.3 D3 discloses immunogenic conjugates of a hapten moiety and γ-D-PGA, which induce an IgM response against the hapten moiety (see the corresponding MEDLINE abstract).

1.4 D4 discloses immunogenic conjugates of glucagon and γ-D-PGA, which elicit cellular and humoral responses to the glucagon moiety (see: abstract; paragraph joining pages 98 and 99).

1.5 D5 and D9 disclose γ-PGA as carrier for drug delivery. In particular, D5 discloses tumour-specific antibodies and anti-cancer agents covalently conjugated to γ-D-PGA moieties from B. licheniformis through thioester linkages (see the abstract). The drug

conjugates disclosed in D9 have a disulphide linkage (see claims 1 and 7). In addition, D9 indicates that PGA drug conjugates account for improved drug pharmacodynamic (see the paragraph joining pages 6 and 7).

1.6 D6 discloses conjugates of PA and γ-D-PGA from B. anthracis for the manufacture of vaccines against Anthrax (see in particular: the abstract; page 8946, left-hand column, second paragraph to the end of the first paragraph on page 8947; table 1). As the priority date is considered to be valid, D6 is not to be taken into account for the purposes of Article 33 PCT.

1.7 D7 and D8 disclose Anthrax vaccines comprising PA as the primary immunogenic antigen (see for example the abstract of D8). Both documents indicate that other antigens from the bacilli are involved in the vaccine immunity, for example sera from immunized individuals cross-reacts with the bacterial spores (see D8: page 1678, left-hand column, lines 7-14; page 1684; right-hand column, lines 24-31). In addition, D7 indicates that antibodies against the bacterial capsule polypeptide γ-D-PGA could also be involved in the immunity to B. anthracis (see page 143, left-hand column, first paragraph). Improved vaccines could therefore contain these additional antigenic specie (see: D7, page 143, right-hand column, first paragraph; D8, paragraph joining pages 1682 and 1683).

2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

2.1 For the assessment of the present claims 17-20 and 31-33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2.2 Claims 1-16 and 21-30 relate to immunogenic conjugates of the capsular polypeptide from B. anthracis and compositions thereof. Said conjugates and compositions can

be made in the pharmaceutical industry, hence they are to be considered as having an industrial applicability according to article 33(4) PCT.

3. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT).

3.1 The subject-matter of claim 1 is not novel over the immunogenic γ-PGA conjugates of D1, D3 and D4 (see points 1.1, 1.3 and 1.4 above). In addition, the subject-matter of claim 1 cannot be considered novel over the γ-PGA-antibody conjugates disclosed in D5 because the immunogenic properties of these conjugates are intrinsic in the antibody component (see point 1.5 above).

3.1[a] The adjective "synthetic" used in claim 1 merely relates to the origin of the γPGA polymer and does not introduce any technical feature, which distinguishes the claimed subject-matter from this prior art (see the PCT Guidelines 5.26). In general, the origin of a product represents a limiting technical feature for claims, which are directed to the preparation and the use of the product (method claims), but does not necessarily represent any limiting technical feature for product claims. In the present case, the broad indication of the synthetic origin of the γPGA polymer does not inherently result in any technical feature of the claimed conjugate. Moreover, γPGA is unambiguously defined in terms of its chemical structure (see for example page 7, lines 16-28, of the application). Accordingly, the distinction between different embodiments is to be based on the structural features of the γPGA polymer, irrespective of its origin. As no distinguishing structural feature is explicitly referred to in claim 1, the γPGA polymers of the prior art fall within the claim scope.

3.2 Dependent claims 2-5, 7, 8, 10-14 and claims 15-20 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, given the disclosure of the prior art (see again points 1.1, 1.3-1.5 above). In particular, medical and pharmaceutical applications of the known γ-PGA conjugates are apparent from the prior art.

3.2[a] With respect to claim 5, it is noted that the list of carrier moieties includes mammalian immunoglobulins as disclosed in D5 and known immunogenic adjuvants.

3.2[b] The additional structural features of claims 2-4, 7, 8 and 10-14 come within the scope

of the customary practice followed by persons skilled in the art and do not apparently lead to any unexpected effect or property, to which an inventive step could be addressed. Hence, the subject-matter of these dependent claims is simply to be considered among the straightforward possibilities, from which the skilled person would select, without the exercise of inventive skill, in order to solve the problem of providing alternative γ-PGA conjugates to the ones disclosed in D1, D3, D4 and D5.

3.3 The subject-matter of claims 6 and 9 is novel over the available prior art in view of the PA moiety conjugated to the γ-PGA polypeptide.

3.3[a] D7 and D8 can be independently considered to represent the relevant state of the art. These documents discloses immunogenic compositions for anthrax vaccines, from which the claimed subject-matter differs in the γ-PGA component covalently conjugated to PA (see point 1.7 above).

3.3[b] The problem to be solved can therefore be regarded as the provision of an improved immunogenic composition for anthrax vaccines.

3.3[c] The prior art explicitly suggests improvements for the anthrax vaccine by incorporating in the PA-based composition additional antigenic epitopes from the bacilli, among which the capsular polypeptide γ-PGA (see again point 1.7 above). The skilled person would therefore have combined the two antigens in order to solve the problem posed, thereby obtaining conjugates according to claims 6 and 9. As indicated above (see points 3.1[a] and 3.2[b]), the synthetic origin of the γ-PGA does not effectively characterize the claimed conjugate. Moreover, the structural features of the covalent linkage and, eventually, the decameric γ-PGA are within the customary practice of the skilled person and do not apparently lead to any unexpected effect or property.

Since D1 teaches the immunogenic properties of γ-PGA in conjugated forms (see point 1.1 above), the skilled person would have considered to solve the problem posed by means of this antigen with a reasonable expectation of success, despite the fact that γ-PGA alone is poorly immunogenic (see D7, page 143, left-hand column, first paragraph).

3.3[d] Independently from the reasoning above, the subject-matter of claims 1-20 cannot be considered to involve any inventive step because it covers embodiments, which do not account for any effective long-lasting immunization (see table 1 of the present application, line 23). Hence, the claimed subject-matter does not solve the problem

posed over the whole claimed scope.

3.4 The subject-matter of claims 21-33 is novel in view of the specific carrier moiety. The available prior art does not disclose any γ-PGA conjugate comprising a carrier moiety selected from the list of claim 21.

3.4[a] Nevertheless, the subject-matter of these claims does not involve any inventive step as explained below.

3.4[b] D1 is considered to represent the relevant state of the art because it discloses immunogenic conjugates of the anthrax γ-PGA polypeptide, from which the subject-matter of claims 21 differs in the carrier moiety.

The problem can therefore be regarded as the provision of alternatives γ-PGA conjugates for producing an immune response against this anthrax antigen.

Since D1 teaches the immunogenic properties of the γ-PGA hapten in conjugated forms, the skilled person would have considered to solve the problem posed by replacing the carrier moiety disclosed in this document with other immunogenic carriers. Most of the carrier moieties listed in claim 21 are among known immunogenic carriers, e.g. serum albumins and KLH. The selection of a specific immunogenic carrier can only be regarded as inventive, if it presents unexpected effects or properties. However, no such effects or properties are indicated in the application, and therefore the subject-matter of claim 21 is not considered to involve any inventive step.

3.4[c] In addition, the subject-matter of claim 21 lacks an inventive step for the same reasons of points 3.3[a]-3.3[d] above.

3.4[d] Dependent claims 22-28 and claims 29-33 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, given the disclosure of the prior art (see also the remarks of points 3.2[b] above).

CLAIMS

1. An immunogenic conjugate comprising a synthetic homopolymer polypeptide of poly-γ-glutamic acid (γPGA) covalently linked to a carrier, wherein the conjugate elicits an immune response in a subject.

2. The conjugate of claim 1, wherein the conjugate comprises a γPGA polypeptide comprising 5-20 glutamic acid residues.

3. The conjugate of claim 1, wherein the conjugate comprises a γPGA polypeptide comprising 10-15 glutamic acid residues.

4. The conjugate of claim 1, wherein the conjugate comprises a decameric γPGA polypeptide.

5. The conjugate of claim 1, wherein the carrier is selected from the group consisting of: (a) bovine serum albumin, (b) recombinant *B. anthracis* protective antigen, (c) recombinant *P. aeruginosa* exotoxin A, (d) tetanus toxoid, (e) diphtheria toxoid, (f) pertussis toxoid, (g) *C. perfringens* toxoid, (h) hepatitis B surface antigen, (i) hepatitis B core antigen, (j) keyhole limpet hemocyanin, (k) horseshoe crab hemocyanin, (l) edestin, (m) mammalian serum albumins, (n) mammalian immunoglobulins, analogs or mimetics of (a)-(n), and combinations thereof.

6. The conjugate of claim 1, wherein the carrier comprises recombinant *B. anthracis* protective antigen.

7. The conjugate of claim 1, wherein the γPGA polypeptide comprises the D- or L-conformation.

8. The conjugate of claim 1, wherein the γPGA polypeptide comprises a γDPGA polypeptide.

9. The conjugate of claim 1, wherein the γPGA polypeptide comprises a decameric γDPGA polypeptide and the carrier comprises recombinant *B. anthracis* protective antigen.

10. The conjugate of claim 1, wherein the carrier is covalently linked to either the amino or carboxyl terminus of the γPGA polypeptide.

11. The conjugate of claim 1, wherein the carrier is covalently linked to the γPGA polypeptide via a thioether, disulfide, or amide bond.

12. The conjugate of claim 1, wherein the density of the γPGA polypeptide to carrier is between about 5:1 and about 32:1.

13. The conjugate of claim 1, wherein the density of the γPGA polypeptide to carrier is between about 10:1 and about 15:1.

14. The conjugate of claim 1, wherein the γPGA polypeptide is covalently linked to the carrier via an aldehyde (CHO)/adipic acid hydrazide (AH) linkage.

15. A composition comprising the conjugate of any one of claims 1-14 and a pharmaceutically acceptable carrier.

16. The composition of claim 15, further comprising an adjuvant.

17. A method of eliciting an immune response against a *Bacillus* antigenic epitope in a subject, comprising introducing into the subject the composition of claim 16, thereby eliciting an immune response in the subject.

18. The method of claim 17, wherein the immune response is elicited against the *Bacillus* capsular poly-γ-glutamic acid (γPGA) polypeptide.

19. The method of claim 17, wherein the immune response is elicited against the *Bacillus* capsular poly-γ-glutamic acid (γPGA) polypeptide and the carrier.

20. The method of claim 17, wherein the immune response comprises opsonophagocytic activity.

21. An immunogenic conjugate comprising a *Bacillus* capsular poly γ glutamic acid (γPGA) polypeptide covalently linked to a carrier, wherein the carrier is selected from the group consisting of: (a) recombinant *B. anthracis* protective antigen, (b) recombinant *P. aeruginosa* exotoxin A, (c) tetanus toxoid, (d) diphtheria toxoid, (e) pertussis toxoid, (f) *C. perfringens* toxoid, (g) hepatitis B surface antigen, (h) hepatitis B core antigen, (i) keyhole limpet hemocyanin, (j) horseshoe crab hemocyanin, (k) edestin, (l) mammalian serum albumins, analogs or mimetics of (a)-(l), and combinations thereof, and wherein the conjugate elicits an immune response in a subject.

22. The conjugate of claim 21, wherein the carrier comprises recombinant *B. anthracis* protective antigen.

23. The conjugate of claim 21, wherein the *Bacillus* capsular γPGA polypeptide comprises a *B. anthracis*, *B. licheniformis*, *B. pumilus*, *or B. subtilis* γPGA polypeptide.

24. The conjugate of claim 21, wherein the *Bacillus* capsular γPGA polypeptide comprises the D- or L-conformation.

25. The conjugate of claim 21, wherein the *Bacillus* capsular γPGA polypeptide comprises a γDPGA polypeptide.

26. The conjugate of claim 21, wherein the carrier is covalently linked to either the amino or carboxyl terminus of the *Bacillus* capsular γPGA polypeptide.

27. The conjugate of claim 21, wherein the carrier is covalently linked to the *Bacillus* capsular γPGA polypeptide via a thioether, disulfide, or amide bond.

28. The conjugate of claim 21, wherein the *Bacillus* capsular γPGA polypeptide is covalently linked to the carrier via an aldehyde (CHO)/adipic acid hydrazide (AH) linkage.

29. A composition comprising the conjugate of any one of claims 21-28 and a pharmaceutically acceptable carrier.

30. The composition of claim 29, further comprising an adjuvant.

31. A method of eliciting an immune response against a *Bacillus* antigenic epitope in a subject, comprising introducing into the subject the composition of claim 30, thereby eliciting an immune response in the subject.

32. The method of claim 31, wherein the immune response is elicited against the *Bacillus* capsular poly-γ-glutamic acid (γPGA) polypeptide.

33. The method of claim 31, wherein the immune response is elicited against the *Bacillus* capsular poly-γ-glutamic acid (γPGA) polypeptide and the carrier.